REMARKS

Reconsideration and continuing examination of the above-identified application is respectfully requested in view of the amendments above and the discussion that follows.

Each of the independent claims has been amended as discussed below. Claims 1-9, 12-17, 19-33, 35-38 and 42-78 are in the case and are before the Examiner.

I. The Amendments

Each of independent claims 1, 18, 42, 63, 75 and 78 has been amended to recite a sequence variation of about 5 percent. Support for these amendments can be found at least at pages 48 and 55 of the specification. Each of claims 18, 42, 63, 75 and 78 has also been amended to recite that the substitutions are conservative as recited in claim 1.

It is thus seen that no new matter has been added.

II. The Action

A. Rejections Under 35 USC §112, Second Paragraph

It is noted with appreciation that the prior rejections based on the second paragraph of Section 112 have been withdrawn.

B. Rejection Under 35 USC §112, First Paragraph

All of the pending claims were again rejected under 35 USC §112, First Paragraph, as allegedly lacking enablement. It is believed that the present amendments have mooted this basis for rejection. However, to the

extent that they may not have done so, this rejection is respectfully traversed.

It cannot be agreed that at the time the parental and present applications were filed that a skilled worker would have any difficulty in making or using any construct of the claims, as that is the requirement under *In re Angstadt and Griffin*, 190 USPQ 214, (CCPA 1976). In *Angstadt*, the Court held that the question of enablement revolves around whether the

> "disclosure contains sufficient teaching regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention" [190 USPQ at 218; emphasis in the original.]

That Court went on to discuss the disclosure that there taught how to make and how to use a claimed catalyst. It continued that if a skilled worker wanted to make another catalyst than those specifically disclosed in the 40 examples that worker could simply follow the disclosure and make a desired catalyst compound. It further pointed out that the catalysis process was not complicated and needed no special conditions nor equipment. The *Angstadt* claims were found to be enabled despite the amazingly large number of catalysts encompassed.

That Court went further in saying that some "experimentation" was permitted and held that the key phrase was "undue", not "experimentation". Practicing of that invention "would not 'require ingenuity beyond that to be expected of one of ordinary skill in the art' ... ", at 218 (citation omitted). The same should be the case here.

In re Wands, 8 USPQ2d 1400, (Fed. Cir. 1988), a case noted in the Action, involved time-consuming

monoclonal antibody preparation and screening, biological and biochemical processes. There, the Court found that practitioners of the art were prepared to screen negative hybridomas. A similar finding was made in *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, (Fed. Cir. 1986). Those familiar with the hybridoma/monoclonal antibody art know that such preparations and screenings often involve months to generate antibodies and thousands of assays. Those procedures are nevertheless well known, accepted and routine in the art.

In the context of the description requirement, the Federal Circuit has held that "an amino acid sequence supports 'the entire genus of DNA sequences' that can encode the amino acid sequence because the 'state of the art has developed' such that it is a routine matter to convert one to the other." [*Capon v Eshhar*, 76 USPQ2d 1078, 1085 (Fed. Cir. 2005) citing *In re Wallach*, 71 USPQ2d 1939 (Fed. Cir. 2004).] It is submitted that the making and using of nucleic acids as are made and used here is no less developed.

The Actions have asserted a lack of guidance, working examples and an unpredictable nature of the art. However, the Actions have provided no evidence of a need for guidance greater than that provided in the specification, nor unpredictability to a degree that would lead to a lack of enablement. In addition, the courts have said working examples are unnecessary [*In re Strahilevitz*, 212 USPQ 561 (CCPA 19982)].

The *Angstadt* catalyst complex molecule contained a transition metal cation from one of several Groups of the Periodic Table, an undisclosed "inorganic anion" for the metal cation, and a hexaalkylphosphoramide whose six alkyl

groups contained one to thirty carbon atoms in each alkyl group. The metal salt (cation plus anion) was said to be present at 1-4 moles per molecule and the hexaalkylphosphoramide was present at 1-8 moles per molecule complex.

Footnote 2 of *Angstadt* noted that the Solicitor asserted that the claim read on thousands of compounds including "any one of at least 50 metal cations combined with any inorganic anion". Actually, "thousands" was a gross underestimate.

For example, there are eight C_1-C_4 alkyl groups; i.e., methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl and iso-butyl. So, for the hexaalkylphosphoramidates where the alkyl groups are C_1-C_4, there are 8^6 or 262,144 different phosphoramidates, omitting possible chiral isomers. Multiplication by the number of cations, anions and ratios (1-4:1 salt and 1-8:1 hexaalkylphosphoramide per molecule) skyrockets the number of compounds just for that relatively small number of alkyl groups.

According to Noller, Chemistry of Organic Compounds, Second ed., W.B. Saunders Company, Philadelphia, 1951, page 38, (copy enclosed as Exhibit 1 and noted on PTO/SB/08A) there are over 4 billion C_{30} alkanes alone. Presuming the number of C_{30} alkyl groups is about the same as the number of alkanes, which is a gross undervaluing as there are 15 straight chain C_{30} alkyls alone, the *Angstadt* formula actually therefore encompassed an astronomical number of separate compounds once all of the anion, cation, alkyl group and ratio permutations encompassed by the claims are taken into account. For example, there would be about $(4 \times 10^9)^6$ or (4096×10^{54}) different C_{30}

hexaalkylphosphoramides alone. That number of compounds exceeds any arbitrarily large number that one could pick from the physical world such as the number of atoms in the earth if it were all iron [(mass = about 6 X 10^{27}g/55.6g/mole) X 6.023 x 10^{23} atoms/mole = about 6.5 X 10^{49} atoms] or the more chemically familiar Avagadro's Number of 6.023 X 10^{23} molecules per mole.

The *Angstadt* inventors disclosed just 40 examples, with one compound that did not work in their process. The Court there held that the inventors did not have to make and test every compound in their claims, nor did every compound have to work.

Angstadt dealt with synthetic organic chemistry. The present application deals with biochemistry relating to HBc chimers. As noted previously, from *Angstadt* and the art already of record, skilled workers knew how to make and use the constructs claimed here at the time the application was filed. It is submitted that given the several examples and citations in the specification the claimed invention was enabled and this basis for rejection should be withdrawn.

The above notwithstanding, the claims have been amended to moot the rejection so as to speed prosecution. It is believed that the previously discussed amendments that recite that a chimer contains "no more than about 5 percent' substitutions in sequence, as well as eliminating variant sequences have made moot this rejection.

The Examiner's attention is also invited to the disclosures of U.S. Patent No. 6,964,769 to Sebbel et al., that recently came to counsel's attention and is noted on enclosed Form PTO/SB/08A. Claim 1 and all of the dependent claims of that presumptively valid patent recite a named

polypeptide sequence "or a sequence having at least 90% sequence identity to said polypeptide sequence . . ." Counsel was unable to find any disclosure as to the identity of the up to 10% substituting residues, as compared to the lower percentage of substitutions in the amended claims herein. It is thus submitted that the present claims define a more limited number of chimers than those of the presumptively valid issued patent that claims similar, but different chimers. It is therefore submitted that Dr. Birkett was in possession of a very large number of the desired chimer molecules as of the filing date, and that this portion of the rejection should be withdrawn.

It is again noted that none of the articles relied-upon or supplied in the prior Actions regarding this rejection contained disclosures specifically related to the hepatitis B core protein, changes in the sequence of that molecule, and possible changes to the resulting function. Those articles are again submitted to be irrelevant to the present claims as the articles teach nothing about the claimed protein whose structure and function are different from each of those proteins discussed in those articles.

The Action's assertions that those articles "prove a basic rule in the protein art that manipulating a protein structure can affect its biological function" are again submitted to be inapplicable here as that so-called "basic rule" is only surmise about what can happen, that is unsupported by art related to HBc. That surmise does not tell us what will in fact invariably occur. As such, it is not predictive. It is not persuasive.

The Action points to the present Example 6 in which results of particle formation with and without a C-terminal cysteine are discussed. The sequence that had

no C-terminal Cys was less stable than the sequence with the C-terminal Cys. Interestingly, that is what is claimed here. The results of Example 6 illustrate that the Action's point is poorly taken, and that the rejection should be withdrawn. On the other hand, should the Examiner have personal knowledge here that backs up that surmise, she is invited to provide it by affidavit or declaration as provided in 37 CFR 1.104(d)(2). Otherwise, this rejection should be withdrawn.

It is thus submitted that the present application contains more than sufficient enablement to permit a worker of ordinary skill at the time of the filling to make and use the invention claimed to the full breadth of the claims without undue experimentation. It is therefore believed and submitted that this basis for rejection should be withdrawn.

C. Rejections Under 35 USC §102(b)

Ireland et al.

Withdrawal of the rejection of claims 1-8, 18, 27-28, 32-33, 42, 63 and 75 as allegedly anticipated by the disclosures of Ireland US Patent No. 5,990,085 is noted with appreciation.

D. Rejection Under 35 USC §103(a)

Pumpens In View Of Zlotnick

Claims 1-9, 12-33, 35-38, 42-78 were again rejected as allegedly obvious over the disclosures of Pumpens (1995), in view of Zlotnick, noted previously. This rejection is again respectfully traversed as discussed hereinbelow.

The present Action dismissed its erroneous prior reliance on the full length chimeras of Pumpens' Table 1 that contain a heterologous epitope by saying that both Pumpens and Zlotnick teach C-terminally truncated molecules and reasons for their truncation. Of course, Pumpens has no stabilization at the C-terminus of a truncated molecule and Zlotnick has neither an inserted epitope, nor a suggestion of where to put one. In addition, neither relied-on disclosure teaches that a particle formed from C-terminal truncated HBc chimers including an inserted sequence has decreased stability relative to a truncated sequence or a full-length 183-Cys-containing sequence that includes an inserted sequence, nor does either article teach what to do about the non-conservative substitutions present in Zlotnick .

The Action next asserts that Zlotnick followed the erroneous statement in Pumpens concerning a stabilizing effect of sequences added internally to C-truncated HBc chimers, but no disclosure is found in Zlotnick concerning any inserted sequence. The combining of the two disclosures is rather the result of a hindsight expedition looking for bits and pieces of unrelated art that could be put together to seem to make up a whole but have no conceptual glue to keep itself together.

The thrust of the Zlotnick disclosure was concerned with the morphology of HBc capsid particles and whether those particles were greater or smaller in size based on the length of the C-terminal region. Those authors found that the "C terminus of the assembly domain (residues 140-149) functions as a morphogenic switch, longer C termini favoring a higher proportion of the larger

capsids, . . ." (Abstract.) It is thus submitted that the Action has highlighted a portion of the Zlotnick teaching that was incidental to that disclosure and used there merely as a control study, taken it out of context, combined it with contrary teachings of Pumpens to arrive at the present claims. In making that hindsight reconstruction, the Action still has the disclosure of Pumpens concerning a stabilizing feature of added internal sequences in C-truncated chimers to dispose of in finding motivation to combine the teachings, and utterly fails to deal with the issue. The Action also fails to deal with the selective substitutions of Cys residues in Zlotnick and how to put back three of the Cys residues while keeping the fourth. This basis for rejection should be withdrawn.

The Action disagreed that the Pumpens and Zlotnick teachings are unrelated by asserting that both were "cited by Applicant in "BACKGROUIND OF THE INVENTION" of the instant application. Both papers were indeed cited in the present specification. They were, however, cited separately and for different purposes. Pumpens was cited on four pages (4, 5, 32 and 49) and Zlotnick only on page 4, with the two page 4 citations being separated by an intervening paragraph. Pumpens was discussed regarding inserted sequences, whereas Zlotnick was cited for his replacement of all of the internal cysteines and addition of a C-terminal Cys to link the protein to gold particles. Indeed, the Zlotnick paper is entitled "Localization of the C terminus of the assembly domain of hepatitis B virus capsid protein: Implications for morphogenesis and organization of encapsidated RNA", and that was what the paper was about. Zlotnick cared about the structure of the HBc particles that he determined by cryo-electron

microscopy, and where the various axes of symmetry were located. His section entitled "CONCLUSIONS AND IMPLICATIONS" says nothing about stability imparted by the added Cys residue at position 150. A skilled worker looking for ways to stabilize a chimer might read the Abstract and all or part of the Conclusions, and would conclude that the article is not relevant to the problem of stabilizing particles. Indeed, the Abstract notes that the gold-labeled "protein is unimpaired in its ability to form capsids."

The inventor here, if anyone, put together the Zlotnick and Pumpens teachings. The art did not combine their teachings, nor was there any reason for the art to do so. It is respectfully submitted that only the inventor's disclosure and not the prior art provides a motive for achieving the combination claimed by the inventor.

To imbue one of ordinary skill in the art with knowledge of the invention when no prior art disclosures convey or suggest that knowledge is to fall victim to the effect of hindsight in which that which the inventor taught is used against him. Of course, it is improper to use the inventor's own teachings to reject claims under the guise of Section 103. [*In re Saniford*, 149 USPQ 301 (CCPA 1966); *In re Dow Chemical*, 5 USPQ 2d 1529 (Fed. Cir. 1988); *In re Vaeck*, 20 USDPQ 2d (Fed. Cir. 1991), cited at MPEP 706.02(k); *Ex parte Giles*, 228 USPQ 866 (BPAI 1988)]

It is reiterated in this context that the before cited and discussed paper by a worker of at least ordinary skill, Ulrich (cited at page 7 of the application and discussed in the previous Reply), stated that the stability problem of HBc was not solved, and Ulrich's citing the relied-on Pumpens paper indicated that the problem of

stability was not deemed by those skilled workers to be solved by inserting a heterologous sequence into the HBc sequence as is suggested by Pumpens. Ulrich also cited the relied-on Zlotnick publication, and not having the present invention laid out before him did not suggest that the desired stability could be achieved by combining those teachings. It is again submitted that this basis for rejection should be withdrawn.

The Examiner's attention on this point is invited to *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579; 27 USPQ2d 1200 (1993). That case dealt with the admissibility of expert testimony and criteria used for admission of that testimony in a trial. The Court noted that the "fact of publication (or lack thereof) in a peer reviewed journal thus will be a relevant, though not dispositive, consideration in assessing the scientific validity of a particular technique or methodology on which an opinion is based." (27 USPQ2d at 1206.)

In discussing evidence provided by an expert witness in "determining the knowledge that a person of ordinary skill in the art would have possessed at a given time," the Federal Circuit recently held that such evidence "is pertinent to our evaluation of a prima facie case of obviousness." *Alza Corp. v. Mylan Laboratories, Inc.*, 06-1019, Slip at 13 (Fed. Cir. Sept. 6, 2006). It is submitted that Ulrich can be deemed such a witness whose testimony is provided in his published writing. As such a witness, his writings should be afforded more weight than the unsupported suppositions as to what may be in the mind of a hypothetical person of ordinary skill. Rather, Ulrich is (was) an author of probably greater than ordinary skill, having published at least 24 papers since 2002 in viral-

related technologies (see, attached Exhibit 2 from a Google search). Ulrich wrote, in a paper that cited both the relied-on Pumpens and Zlotnick papers, that a possible problem of chimer usage in vaccines related to the requirement of reproducible preparation of intact chimer particles that were stable and could withstand long-term storage.

Ulrich published that paper in a peer reviewed journal, was (is) a worker of at least ordinary skill in this art, whose paper is relied-on in this Action, cited both Zlotnick and Pumpens and was therefore aware of their contents. Nonetheless, Ulrich did not put together the combination of the two teachings to solve the stability problem that he wrote about, but rather maintained that the problem still had to be solved. It is submitted that if Ulrich the real, live worker of more than ordinary skill working and writing in this art did not put together the relied-on art as has the hypothetical skilled worker of the Action, the Action is mistaken in its conclusion as to the abilities of its hypothetical worker and obviousness, and that conclusion of obviousness should be withdrawn.

The Examiner is thanked for citing the portion of the Pumpens disclosure relating to "linkers". It is important to note that the statement now relied-on recites that the "short polylinkers . . . code for . . . well-characterized epitopes . . . " (emphasis supplied). Thus, indeed, the linkers are nucleic acid sequences that code for a peptide sequence. Those linkers are not "a heterologous linker residue for a conjugated epitope" as recited in the claims and described in the specification. Those linkers are not a proper basis for rejection as recited in the prior action. The fact that the polylinker

codes for a well-recognized epitope is irrelevant to the claims as that was not the basis for the original rejection.

Further to this point, the undersigned searched the Google search engine under the query "polylinker", and Google noted 443,000 hits. The first page of those search results is attached as Exhibit 3 for the Examiner's convenience. It will be seen that the word "polylinker" means a nucleic acid sequence.

In Paragraph 17, the Action relies on the decision in *In re Keller* that held that the test for obviousness is not whether the features of a secondary reference may be bodily incorporated in the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art.

The Court discussed *In re Keller* in *In re Fine*, 5 USPQ2d 1596, 1599 (Fed. Cir. 1988) that is also cited in the Action. The *Fine* case related to the substitution of one type of gas detector for another detector, and was thus not unlike *Keller* on the facts. However, the Court, after quoting from *Keller*, stated:

> [b]ut [obviousness] "cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination". [Citation omitted.] And "teachings of references can be combined *only* if there is some suggestion or incentive to do so." [Citation omitted.] Here,

> the prior art contains none. (Emphasis in the original.)

It is respectfully submitted that the same holds here. There is no prior art teaching or suggestion supporting the combination made in the Action. This basis for rejection should be withdrawn.

The Action asserts in Paragraph 20 that "Zlotnick has provided the knowledge of minimal determinant of HBV capsid assembly, which is important for the art of basic and applied HBV virology, because 'some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references.'" (Quoting from *Fine*, above.) It is submitted that neither the first part of the above quote nor the part quoted from *Fine* is correct or correctly taken in context.

If the phrase "minimal determinant of HBV capsid assembly" were intended to mean that Zlotnick was the first to define the minimal sequence length requirements for particle formation, it is submitted that the paragraph bridging pages 4 and 5 of this application points out that those requirements were noted in the relied-on Pumpens paper two years before Zlotnick was published. It must also be remembered that the Pumpens paper was more of a review article than an original research article, so it is likely that others before Pumpens worked out those parameters. Thus, even though Zlotnick's paper title purported to relate to "Localization of the C terminus of the assembly domain of hepatitis B virus capsid protein . . . " others had hoed that row years before, and as pointed out in Pumpens, the art already knew where the C-terminal

end of the assembly domain was. Rather, it is believed that Zlotnick meant that he had found a determining factor in whether the capsids had T=3 or T=4 symmetry, a point not at issue here.

Second, the quote from *Fine* was taken from the portion of that case that discussed the requirements for the PTO's burden in establishing a *prime facie* case of obviousness. In *Fine* as here, the PTO did not carry its burden, and this rejection should be withdrawn.

Paragraph 22 of the Action now asserts that one would combine the teachings of Pumpens with those of Zlotnick

> because it was well known that HBc chimeras with C-terminal deletions, while still capable of self assembly, were less stable that their full length counterparts and that by adding back amino acid residues to these C-terminal deletion[s] one could achieve a more stable chimers, while Zlotnick teaches that the addition of a cysteine to an HBc C-terminal truncation results in enhanced stability.

That statement cannot be agreed with on several levels. It can be agreed, however, that it was well known that "chimeras with C-terminal deletions, while still capable of self assembly, were less stable that their full length counterparts".

It is submitted that there are no teachings of record that additions of C-terminal residues in excess of those recited in the claims enhances stability. Rather, Zlotnick teaches that elongation of the C-terminus favors formation of larger capsid particles. (Summary) The

Examiner is again asked to more precisely indicate the art on which she is relying if this rejection is maintained.

The above-quoted statement also totally disregards the disclosure at Pumpens page 67, left side (underlined) that states:

> [a]lthough capsids formed by C-terminally truncated HBc monomers are less stable than the corresponding full-length protein particles [citations omitted], foreign insertions are not only possible but also exert a stabilizing effect on HBcΔ derivatives, especially in the case of internal insertions [citation to Borisova's unpublished work omitted].

The question must again be asked, "Why would a skilled worker combine the teachings of Pumpens and Zlotnick when Pumpens teaches that one can gain stability in C-terminally truncated chimers by having internal insertions? The answer is that such a worker would not do so.

Thus, the skilled worker at the time the invention here was made, trying to obtain the stabilized HBc chimer particle Ulrich said would be useful, would not have combined the teachings of Pumpens with those of Zlotnick. First, that worker would have heeded Pumpens who taught that adding internal sequences to C-terminal polypeptides provided enhanced stability. Next, once it was found out that Pumpens was incorrect in the statement, that worker still would not have gone to the Zlotnick paper because its title, Abstract and Conclusions all provide no implication that it might be useful. Further reading of Zlotnick would also provide no inkling that its teachings should be combined with those of Pumpens, because Zlotnick

teaches nothing about the effects that might be obtained when insertions were added to the truncated protein, to say nothing about not mutating the Cys residues at positions 48, 61 and 107 into non-conservative substitutions. Actually, the skilled worker would read the later published Ulrich article and learn that the stability issue had not yet been solved and would therefore understand why the presently claimed invention is not obvious and why this rejection should be withdrawn.

F. Summary

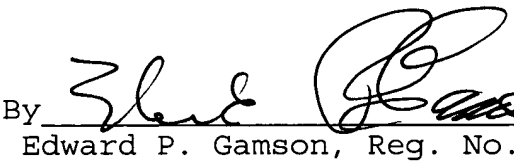
Claims 1, 18, 51, 63 and 75 have been amended. Each of the bases for rejection has been dealt with and overcome or otherwise made moot.

It is therefore believed that this application is in condition for allowance of all of the pending claims. An early notice to that effect is earnestly solicited.

A Petition for an Extension Of time and its fee are enclosed to permit the Examiner further time to deal with this paper. No further fee or petition is believed to be necessary. However, should any further fee be needed, please charge our Deposit Account No. 23-0920, and deem this paper to be the required petition.

The Examiner is requested to phone the undersigned should any questions arise that can be dealt with over the phone to expedite this prosecution.

Respectfully submitted,

By 
Edward P. Gamson, Reg. No. 29,381

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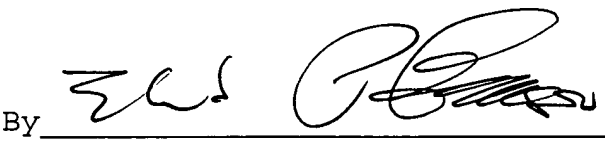
Enclosures
Exhibits 1 through 3
Petition and fee
Form PTO/SB/08A

CERTIFICATE OF MAILING

I hereby certify that this Reply and its stated enclosures are being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: MAIL STOP AF, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450, on October 5, 2006.

By 
Edward P. Gamson

Chemistry of Organic Compounds

CARL R. NOLLER
Professor of Chemistry, Stanford University

SECOND EDITION

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for example, would be called 2-methyl-5-(1,2-dimethylpropyl)nonane. The first five compounds in Table 5 are all hexanes because they have six carbon atoms, regardless of the fact that they are named as derivatives of methane, butane, and pentane. The last compound of the list is a pentadecane even though it is named as a derivative of nonane.

In the naming of compounds as derivatives of another compound, it is conventional to consider the name as one word rather than to write the names of groups and parent compound as separate words or to use an unnecessary number of hyphens. It is preferable to limit the use of hyphens to the attachment of position numbers and symbols.

As the number of carbon atoms increases, the number of structural isomers possible soon reaches astronomical proportions as shown by the following figures which were arrived at by rather complicated mathematical formulas.

C_7—9	C_{15}—4347
C_8—18	C_{20}—366,319
C_9—35	C_{30}—4,111,846,763
C_{10}—75	C_{40}—6.25×10^{13}

By 1947 all of the predicted alkanes through the nonanes, and over half of the decanes were known. Only a few isomers of each of the higher groups of compounds are known, chiefly because there has not been a sufficiently good reason for organic chemists to attempt to synthesize them or because those who are interested in them have not yet had time to do so. The largest alkane of known structure and molecular weight synthesized so far has the molecular formula $C_{100}H_{202}$.

Physical Properties

The physical properties of organic compounds depend in general on the number and kind of atoms in the molecule and on the way in which the atoms are linked together. At 25° and 760 mm., the normal hydrocarbons are gases from C_1 to C_4, liquids from C_5 to C_{17}, and solids for C_{18} and above.

Boiling Points. The boiling points of the normal hydrocarbons increase with increasing molecular weight. When plotted against the number of carbon atoms, they fall on a smooth curve as shown in Fig. 26. The rise in boiling point is due to the increased attraction between molecules as the number of atoms increases. This attraction is known as the *van der Waals force*[6] and comes into play when molecules are close to each other.

Branching of the chain always results in a lowering of the boiling point, the van der Waals force being smaller for a more compact molecule than for a longer molecule. Thus *n*-pentane boils at 36°, isopentane at 28°, and neopentane at 9.5°.

[6] So-called because it is the cause of the a term in the van der Waals equation, $\left(P + \frac{a}{V^2}\right)(V - b) = RT$ for one mole of gas. The law for a perfect gas, $PV = RT$, holds only if the molecules of a gas have no attraction for each other and occupy no volume. By introducing a constant a to take care of the attraction between molecules and a constant b to offset the volume occupied by the molecules, the equation fits more closely the experimental data. Constants a and b are characteristic for each compound.

Ulrich R (Rainer)

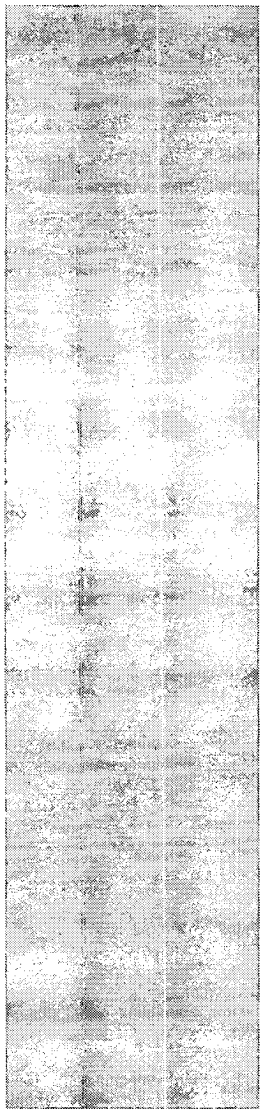
papers: 24

Virus Genes. 2006 Aug 22;:

Hamster polyomavirus-derived virus-like particles are able to transfer in vitro encapsidated plasmid DNA to mammalian cells.

Tatyana Voronkova, Andris Kazaks, Velta Ose, Muhsin Ozel, Siegfried Scherneck, Paul Pumpens, Rainer Ulrich

The authentic major capsid protein 1 (VP1) of hamster polyomavirus (HaPyV) consists of 384 amino acid (aa) residues (42 kDa). Expression from an additional in-frame initiation codon located upstream from the authentic VP1 open reading frame (at position -4) might result in the synthesis of a 388 aa-long, amino-terminally extended VP1 (aa -4 to aa 384; VP1(ext)). In a plasmid-mediated Drosophila Schneider (S2) cell expression system, both VP1 derivatives as well as a VP1(ext) variant with an amino acid exchange of the authentic Met1Gly (VP1(ext-M1)) were expressed to a similar high level. Although all three proteins were detected in nuclear as well as cytoplasmic fractions, formation of virus-like particles (VLPs) was observed exclusively in the nucleus as confirmed by negative staining electron microscopy. The use of a tryptophan promoter-driven Escherichia coli expression system resulted in the efficient synthesis of VP1 and VP1(ext) and formation of VLPs. In addition, establishment of an in vitro disassembly/reassembly system allowed the encapsidation of plasmid DNA into VLPs. Encapsidated DNA was found to be protected against the action of DNase I. Mammalian COS-7 and CHO cells were transfected with HaPyV-VP1-VLPs carrying a plasmid encoding enhanced green fluorescent protein (eGFP). In both cell lines eGFP expression was detected indicating successful transfer of the plasmid into the cells, though at a still low level. Cesium chloride gradient centrifugation allowed the separation of VLPs with encapsidated DNA from "empty" VLPs, which might be



useful for further optimization of transfection. Therefore, heterologously expressed HaPyV-VP1 may represent a promising alternative carrier for foreign DNA in gene transfer applications.

Virology. 2006 Aug 9;:

[Pubmed] [Scholar] [Select] [Hide]

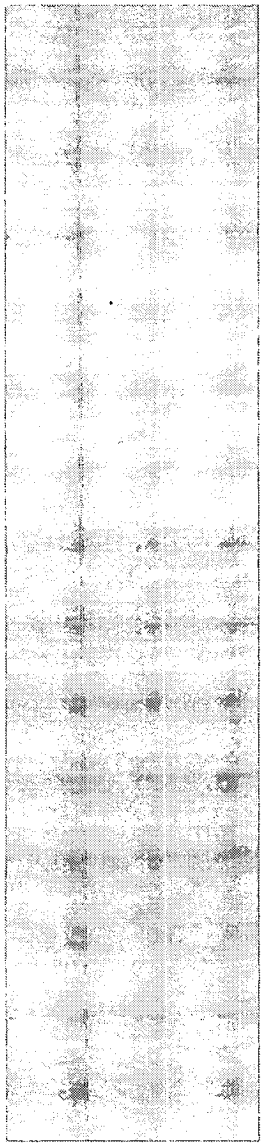
Virus-like particles derived from major capsid protein VP1 of different polyomaviruses differ in their ability to induce maturation in human dendritic cells.

Alma Gedvilaite, David C Dorn, Kestutis Sasnauskas, Gabriele Pecher, Aiste Bulavaite, Robert Lawatscheck, Juozas Staniulis, Tina Dalianis, Torbjörn Ramqvist, Günther Schönrich, Martin J Raftery, Rainer Ulrich

As polyomavirus major capsid protein VP1-derived virus-like particles (VLPs) have been demonstrated to be highly immunogenic, we studied their interaction with human dendritic cells (hDCs). Exposure of hDCs to VLPs originating from murine (MPyV) or hamster polyomavirus (HaPyV) induced hDC maturation. In contrast, exposure of hDCs to VLPs derived from human polyomaviruses (BK and JC) and simian virus 40 (SV40) only marginally induced DC maturation. The hDCs stimulated by HaPyV- or MPyV-derived VLPs readily produced interleukin-12 and stimulated CD8-positive T-cell responses in vitro. The highest frequencies of activated T cells were again observed after pulsing with HaPyV- and MPyV-derived VLPs. Monocyte-derived hDCs both bound and internalized the various tested polyomavirus VP1-derived VLPs with different levels of efficiency, partially explaining their individual maturation potentials. In conclusion, our data suggest a high variability in uptake of polyomavirus-derived VLPs and potency to induce hDC maturation.

Virus Res. 2006 Jun 14;:

[Pubmed] [Scholar] [Select] [Hide]



Generation of virus-like particles consisting of the major capsid protein VP1 of goose hemorrhagic polyomavirus and their application in serological tests.

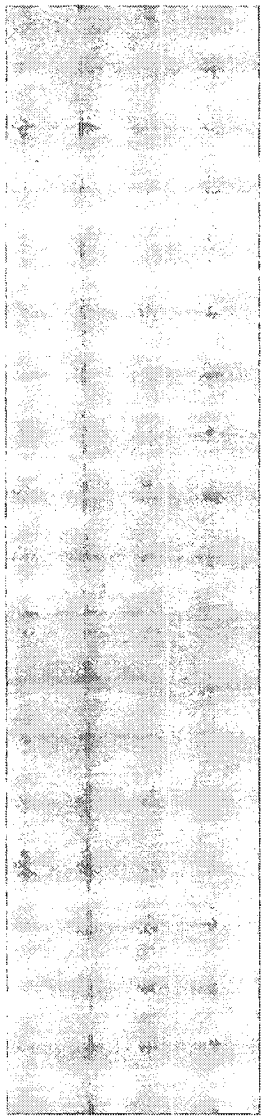
Anja Zielonka, Alma Gedvilaite, Rainer Ulrich, Dörte Lüschow, Kestutis Sasnauskas, Hermann Müller, Reimar Johne

Goose hemorrhagic polyomavirus (GHPV) is the causative agent of hemorrhagic nephritis and enteritis of geese (HNEG), a fatal disease of young geese with high mortality rates. GHPV cannot be efficiently propagated in tissue culture. To provide antigens for diagnostic tests and vaccines, its major structural protein VP1 was recombinantly expressed in Sf9 insect cells and in the yeast Saccharomyces cerevisiae. As demonstrated by density gradient centrifugation and electron microscopy, GHPV-VP1 expressed in insect cells formed virus-like particles (VLPs) with a diameter of 45nm indistinguishable from infectious polyomavirus particles. However, efficiency of VLP formation was low as compared to the monkey polyomavirus SV-40-VP1. In yeast cells, GHPV-VP1 alone formed smaller VLPs, 20nm in diameter. Remarkably, co-expression of GHPV-VP2 resulted in VLPs with a diameter of 45nm. All three types of GHPV-VLPs were shown to hemagglutinate chicken erythrocytes. ELISA and hemagglutination inhibition tests using the VLPs as antigen detected GHPV-specific antibodies in up to 85.7% of sera derived from flocks with HNEG but in none of the sera of a clinically healthy flock. However, GHPV-specific antibodies were also detected in sera from two other flocks without HNEG indicating a broad distribution of GHPV due to subclinical or unrecognised infections.

J Clin Microbiol. 2006 Apr ;44:1608-11

Saaremaa hantavirus should not be confused with its dangerous relative, dobrava virus.

Alexander Plyusnin, Antti Vaheri, Ake Lundkvist, Boris Klempa, Helga Meisel, Detlev H Krüger, Rainer Ulrich, Michal Stanko, Milan Labuda



J Virol Methods. 2006 Feb 25;:

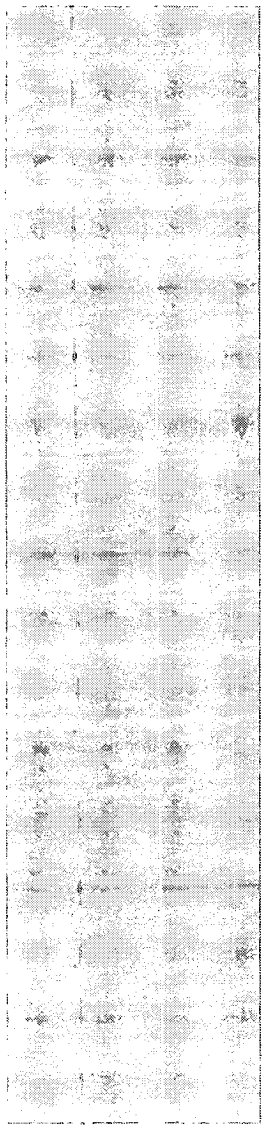
A novel method for cloning of non-cytolytic viruses.

Andreas Rang, Harald Heider, Rainer Ulrich, Detlev H Krüger

Hantaviruses are rodent-borne pathogens with a segmented single-stranded RNA genome of negative polarity. Spontaneous occurrence of variants with genetic heterogeneity have been observed both in vivo and in vitro. The objective of this study was to establish a method for the cloning of genetically homogenous hantaviruses which can be used for subsequent functional studies. Infected VeroE6 cells were incubated with an agarose/medium overlay to prevent uncontrolled distribution of de novo synthesized virus. Thereafter, the overlay was removed and stored for isolation of the diffused virus. The cell layer was fixed and viral antigen-containing foci were detected by immunochemistry. The relative location of the foci on the culture dish was used to trap individual virus clones in the corresponding overlay. The clones were picked and used for re-infection. According to this novel protocol three different hantaviruses, i.e. Hantaan, Puumala, and Tula virus, were purified. In the course of purification the titers of the resulting virus stocks were increased by 10-1000-fold. In addition, this method was used to purify a minor Puumala virus variant from a parental stock containing a mixture of two variants. Taken together, the method presented is well suited to isolate genetically homogenous hantaviruses and might also be applicable for other non-cytolytic viruses.

Intervirology. 2006 ;49:173-84

Serological assays for the detection of human andes hantavirus infections based on its yeast-expressed nucleocapsid protein.



Jonas Schmidt, Helga Meisel, Silvana G Capria, Rasa Petraityte, Ake Lundkvist, Brian Hjelle, Pablo A Vial, Paula Padula, Detlev H Kruger, Rainer Ulrich

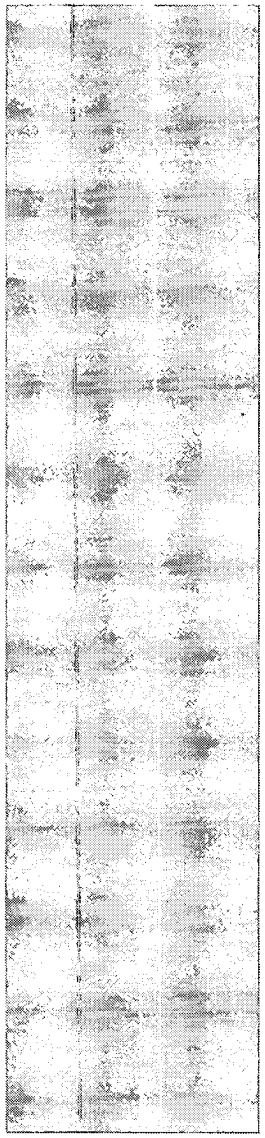
Background: The objective of the study was to develop and evaluate IgM and IgG ELISAs and an IgG Western blot test for the serological detection of human infections with Andes virus (ANDV), the major cause of hantavirus cardiopulmonary syndrome (HCPS) in South America. Methods: The entire nucleocapsid (N) protein-encoding sequence of ANDV (strain AH-1) was cloned and expressed in the yeast Saccharomyces cerevisiae. The polyhistidine-tagged recombinant N (rN) protein of ANDV was purified by nickel-chelation chromatography and characterized by its reactivity with different N-specific monoclonal antibodies. To detect an antibody response directed against ANDV in humans, indirect IgM and IgG ELISAs and an IgG Western blot test based on ANDV rN antigen were developed. The evaluation of the tests was performed using a negative serum panel and 63 blinded sera from Argentina and Chile, containing acute-phase and convalescent sera from HCPS patients. Results: The specificities and sensitivities for the IgM and IgG ELISAs were demonstrated to be very high. The IgG ELISA data were confirmed by the IgG Western blot assay based on the same rN antigen. Almost all anti-ANDV-positive sera reacted to higher endpoint titers with N protein of ANDV than with those of Sin Nombre, Laguna Negra or Puumala virus. The cross-reactivity of anti-ANDV-N IgG-positive sera to rN proteins of other hantaviruses was found to be increased with time after the onset of HCPS. Conclusion: The high sensitivity of the novel assays should facilitate early diagnosis of ANDV infections and might contribute to a successful treatment of HCPS patients. Copyright (c) 2006 S. Karger AG, Basel.

J Clin Microbiol. 2005 Jun ;43:2756-63

Central European Dobrava Hantavirus isolate from a striped field mouse (Apodemus agrarius).

Boris Klempa, Michal Stanko, Milan Labuda, Rainer Ulrich, Helga Meisel, Detlev H Krüger

Dobrava virus (DOBV) is a hantavirus that causes hemorrhagic fever with renal syndrome (HFRS) in Europe. It is hosted by at least two



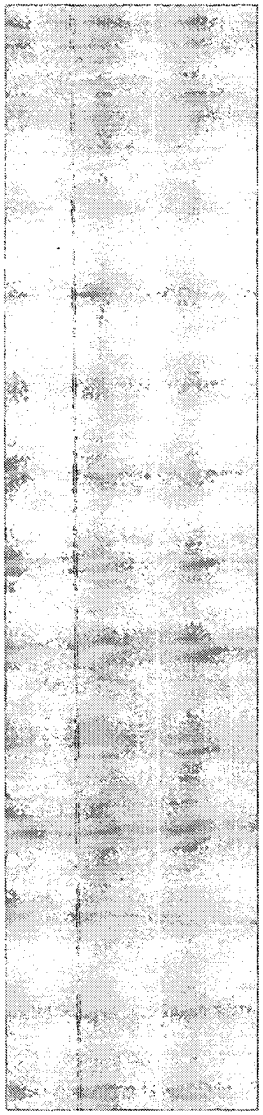
rodent species, Apodemus flavicollis and A. agrarius. According to their natural hosts they form the distinct genetic lineages DOBV-Af and DOBV-Aa, respectively. We have now established a DOBV isolate named Slovakia (SK/Aa) from an A. agrarius animal captured in Slovakia. The complete S and M and partial L segment nucleotide sequences of the new isolate were determined. Phylogenetic analyses showed that the SK/Aa isolate clustered together with the other DOBV-Aa sequences amplified from A. agrarius before and can be taken as the representative of this genetic lineage. SK/Aa, in comparison with a DOBV-Af isolate, was used for serotyping neutralizing antibodies of HFRS patients in Central Europe. Most patients' sera exhibited a higher endpoint titer when probed with our new isolate, suggesting that DOBV-Aa strains are responsible for most of the DOBV-caused HFRS cases in this region.

Intervirology. ;48:255-61

Inactivation of Hantaan virus-containing samples for subsequent investigations outside biosafety level 3 facilities.

Annette A Kraus, Christina Priemer, Harald Heider, Detlev H Kruger, Rainer Ulrich

OBJECTIVES: The potential risk of accidental infection by hantaviruses in a clinical or research laboratory necessitates special precautionary measures. A biosafety program must address handling and disposal of infectious materials as well as appropriate virus inactivation or depletion procedures to permit necessary further processing of specimens outside the biosafety level 3 laboratory. METHODS: To study the elimination of hantavirus infectivity, the effects of different chemical and physical inactivation and depletion procedures were investigated on Hantaan virus-containing materials. An infectivity assay for hantaviruses was utilised to verify these procedures which are commonly preceding investigations such as ELISA, flow cytometry analysis, Western blot or immunofluorescence assay. RESULTS: Chemical inactivation with methanol, paraformaldehyde, acetone/methanol and detergent-containing lysis buffer as well as physical forces such as UV irradiation and filtration efficiently reduced viral infectivity in infected cells and their supernatants



below the detection limit. CONCLUSION: The virus inactivation and depletion methods described herein are suitable to prepare non-infectious samples for further use in immunological, virological and cell-biological assays.

Vaccine. 2005 Jun 10;23:3973-83

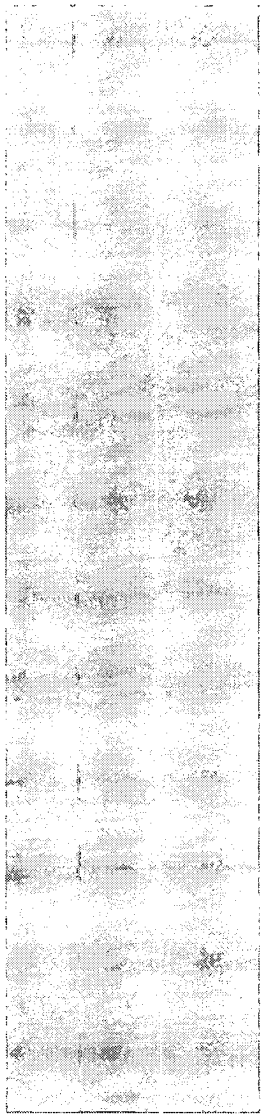
A hantavirus nucleocapsid protein segment exposed on hepatitis B virus core particles is highly immunogenic in mice when applied without adjuvants or in the presence of pre-existing anti-core antibodies.

Astrid Geldmacher, Dace Skrastina, Galina Borisova, Ivars Petrovskis, Detlev H Krüger, Paul Pumpens, Rainer Ulrich

Hepatitis B virus (HBV) core particles carrying the amino-terminal 120 amino acids (aa) of the nucleocapsid (N) protein of the hantaviruses Dobrava, Hantaan or Puumala have been demonstrated to be highly immunogenic in mice when complexed with adjuvants. Here we demonstrate that even without adjuvant, these chimeric particles induced high-titered, and strongly cross-reactive N-specific antibody responses in BALB/c and C57BL/6 mice. The induced N-specific antibodies represented all IgG subclasses. Pre-existing core-specific antibodies did not abrogate the induction of an N-specific immune response by a hantavirus N insert presented on core particles. Therefore, chimeric core particles should represent promising vaccine candidates even for anti-core positive humans.

J Clin Virol. 2005 Jul ;33:247-53

Development and evaluation of serological assays for detection of human hantavirus infections caused by Sin Nombre virus.

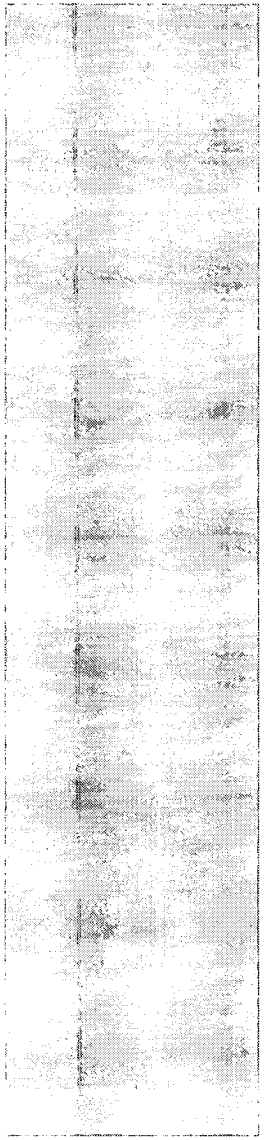


Jonas Schmidt, Helga Meisel, Brian Hjelle, Detlev H Krüger, Rainer Ulrich

BACKGROUND: The hantavirus cardiopulmonary syndrome (HCPS) was first recognized in 1993 after a cluster of acute respiratory distress syndrome deaths in the southwestern of the United States. The major causative agent of HCPS in North America is the Sin Nombre virus (SNV) carried by the deer mouse Peromyscus maniculatus. The first HCPS case imported to Europe was reported in 2002. OBJECTIVES: The objective of the study was to develop and evaluate ELISA and Western blot tests for the serological detection of human infections caused by SNV including those imported to Europe. STUDY DESIGN: A polyhistidine (His)-tagged recombinant nucleocapsid (rN) protein of SNV was expressed in Saccharomyces cerevisiae and purified by nickel chelation chromatography. On the basis of the purified SNV rN protein mu-capture and indirect IgM and IgG ELISAs and an IgG Western blot were developed. The evaluation of the tests was performed using a negative serum panel and a blinded serum panel from the US containing acute-phase sera from HCPS patients. RESULTS: Based upon the results obtained using a panel of negative control sera the specificity for SNV mu-capture and indirect IgM and IgG ELISAs were found to be 100%. All 33 sera from SNV-infected HCPS patients included in the blinded panel were detected by the SNV mu-capture and indirect IgM ELISAs. Twenty-nine out of the 33 SNV-IgM positive sera reacted also in the SNV-IgG ELISA. An SNV-IgG Western blot confirmed the data of the SNV-IgG ELISA. Although the majority of anti-SNV positive sera cross-reacted with rN proteins of Puumala virus and Dobrava virus, the lacking reactivity of a few sera with these heterologous rN antigens in the corresponding IgM and IgG ELISAs demonstrates the value of virus-specific test formats for acute-phase sera. CONCLUSIONS: The novel SNV ELISA and Western blot tests represent a useful tool for the serological detection of SNV infections.

Virus Genes. 2005 Jan ;30:37-48

Nucleocapsid protein of cell culture-adapted Seoul virus strain 80-39: analysis of its encoding sequence, expression in yeast and immuno-reactivity.



Jonas Schmidt, Burkhard Jandrig, Boris Klempa, Kumiko Yoshimatsu, Jiro Arikawa, Helga Meisel, Matthias Niedrig, Christian Pitra, Detlev H Krüger, Rainer Ulrich

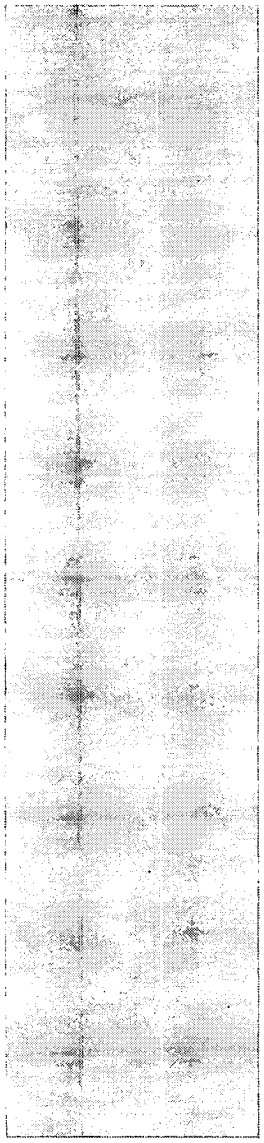
Seoul virus (SEOV) is a hantavirus causing a mild to moderate form of hemorrhagic fever with renal syndrome that is distributed mainly in Asia. The nucleocapsid (N) protein-encoding sequence of SEOV (strain 80-39) was RT-PCR-amplified and cloned into a yeast expression vector containing a galactose-inducible promoter. A survey of the pattern of synonymous codon preferences for a total of 22 N protein-encoding hantavirus genes including 13 of SEOV strains revealed that there is minor variation in codon usage by the same gene in different viral genomes. Introduction of the expression plasmid into yeast Saccharomyces cerevisiae resulted in the high-level expression of a hexahistidine-tagged N protein derivative. The nickel-chelation chromatography purified, yeast-expressed SEOV N protein reacted in the immunoblot with a SEOV-specific monoclonal antibody and certain HTNV- and PUUV-cross-reactive monoclonal antibodies. The immunization of a rabbit with the recombinant N protein resulted in the induction of a high-titered antibody response. In ELISA studies, the N protein was able to detect antibodies in sera of experimentally infected laboratory rats and in human anti-hantavirus-positive sera or serum pools of patients from different geographical origin. The yeast-expressed SEOV N protein represents a promising antigen for development of diagnostic tools in serology, sero prevalence studies and vaccine development.

J Biotechnol. 2004 Aug 5;111:319-33

High yields of stable and highly pure nucleocapsid proteins of different hantaviruses can be generated in the yeast Saccharomyces cerevisiae.

Ausra Razanskiene, Jonas Schmidt, Astrid Geldmacher, Andreas Ritzi, Matthias Niedrig, Ake Lundkvist, Detlev H Krüger, Helga Meisel, Kestutis Sasnauskas, Rainer Ulrich

Recently, the high-level expression of authentic and hexahistidine (His)-tagged Puumala (strain Vranica/Hällnäs) hantavirus nucleocapsid protein derivatives in the yeast Saccharomyces cerevisiae has been reported [Dargeviciute et al., Vaccine, 20 (2002) 3523-



3531]. Here we describe the expression of His-tagged nucleocapsid proteins of other Puumala virus strains (Sotkamo, Kazan) as well as Dobrava (strains Slovenia and Slovakia) and Hantaan (strain Fojnica) hantaviruses using the same system. All nucleocapsid proteins were expressed in the yeast S. cerevisiae at high levels. The nucleocapsid proteins can be easily purified by nickel chelate chromatography; the yield for all nucleocapsid proteins ranged from 0.5 to 1.5 mg per g wet weight of yeast cells. In general, long-term storage of all nucleocapsid proteins without degradation can be obtained by storage in PBS at -20 degrees C or lyophilization. The nucleocapsid protein of Puumala virus (strain Vranica/Hällnäs) was demonstrated to contain only traces of less than 10 pg nucleic acid contamination per 100 microg of protein. The yeast-expressed nucleocapsid proteins of Hantaan, Puumala and Dobrava viruses described here represent useful tools for serological hantavirus diagnostics and for vaccine development.

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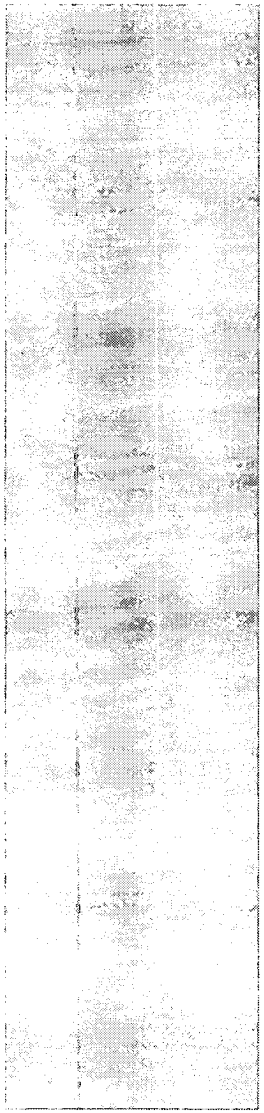
Virology. 2004 May 20;323:108-19

An amino-terminal segment of hantavirus nucleocapsid protein presented on hepatitis B virus core particles induces a strong and highly cross-reactive antibody response in mice.

Astrid Geldmacher, Dace Skrastina, Ivars Petrovskis, Galina Borisova, John A Berriman, Alan M Roseman, R Anthony Crowther, Jan Fischer, Shamil Musema, Hans R Gelderblom, Ake Lundkvist, Regina Renhofa, Velta Ose, Detlev H Krüger, Paul Pumpens, Rainer Ulrich

Previously, we have demonstrated that hepatitis B virus (HBV) core particles tolerate the insertion of the amino-terminal 120 amino acids (aa) of the Puumala hantavirus nucleocapsid (N) protein. Here, we demonstrate that the insertion of 120 amino-terminal aa of N proteins from highly virulent Dobrava and Hantaan hantaviruses allows the formation of chimeric core particles. These particles expose the inserted foreign protein segments, at least in part, on their surface. Analysis by electron cryomicroscopy of chimeric particles harbouring the Puumala virus (PUUV) N segment revealed 90% T = 3 and 10% T = 4 shells. A map computed from T = 3 shells shows additional density splaying out from the tips of the spikes producing the effect of an extra shell of density at an outer radius compared with wild-type



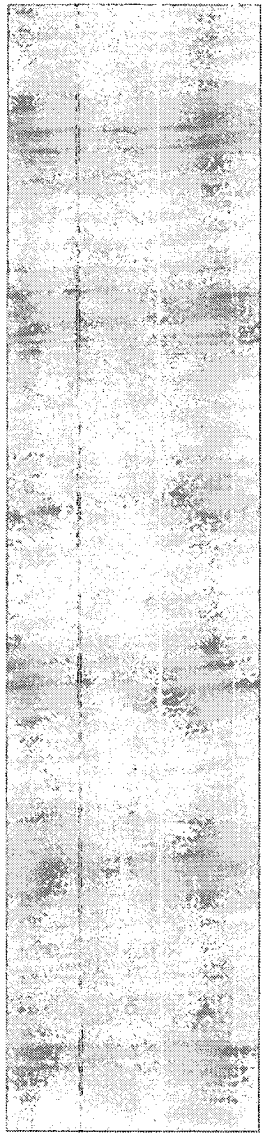
shells. The inserted Puumala virus N protein segment is flexibly linked to the core spikes and only partially icosahedrally ordered. Immunisation of mice of two different haplotypes (BALB/c and C57BL/6) with chimeric core particles induces a high-titered and highly cross-reactive N-specific antibody response in both mice strains.

J Virol. 2004 Jun ;78:6143-50

Differential antiviral response of endothelial cells after infection with pathogenic and nonpathogenic hantaviruses.

Annette A Kraus, Martin J Raftery, Thomas Giese, Rainer Ulrich, Rainer Zawatzky, Stefan Hippenstiel, Norbert Suttorp, Detlev H Krüger, Günther Schönrich

Hantaviruses represent important human pathogens and can induce hemorrhagic fever with renal syndrome (HFRS), which is characterized by endothelial dysfunction. Both pathogenic and nonpathogenic hantaviruses replicate without causing any apparent cytopathic effect, suggesting that immunopathological mechanisms play an important role in pathogenesis. We compared the antiviral responses triggered by Hantaan virus (HTNV), a pathogenic hantavirus associated with HFRS, and Tula virus (TULV), a rather nonpathogenic hantavirus, in human umbilical vein endothelial cells (HUVECs). Both HTNV- and TULV-infected cells showed increased levels of molecules involved in antigen presentation. However, TULV-infected HUVECs upregulated HLA class I molecules more rapidly. Interestingly, HTNV clearly induced the production of beta interferon (IFN-beta), whereas expression of this cytokine was barely detectable in the supernatant or in extracts from TULV-infected HUVECs. Nevertheless, the upregulation of HLA class I on both TULV- and HTNV-infected cells could be blocked by neutralizing anti-IFN-beta antibodies. Most strikingly, the antiviral MxA protein, which interferes with hantavirus replication, was already induced 16 h after infection with TULV. In contrast, HTNV-infected HUVECs showed no expression of MxA until 48 h postinfection. In accordance with the kinetics of MxA expression, TULV replicated only inefficiently in



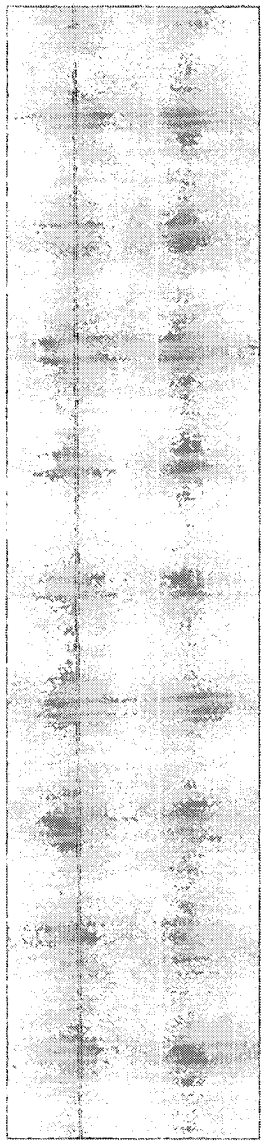
HUVECs, whereas HTNV-infected cells produced high titers of virus particles that decreased after 48 h postinfection. Both hantavirus species, however, could replicate equally well in Vero E6 cells, which lack an IFN-induced MxA response. Thus, delayed induction of antiviral MxA in endothelial cells after infection with HTNV could allow viral dissemination and contribute to the pathogenesis leading to HFRS.

Viral Immunol. ;17:51-68

Segments of puumala hantavirus nucleocapsid protein inserted into chimeric polyomavirus-derived virus-like particles induce a strong immune response in mice.

Alma Gedvilaite, Aurelija Zvirbliene, Juozas Staniulis, Kestutis Sasnauskas, Detlev H Krüger, Rainer Ulrich

Insertion of a short-sized epitope at four different sites of yeast-expressed hamster polyomavirus major capsid protein VP1 has been found to result in the formation of chimeric virus-like particles. Here, we demonstrate that the insertion of 45 or 120 amino acid-long segments from the N-terminus of Puumala hantavirus nucleocapsid protein into sites 1 (amino acids 80-89) and 4 (amino acids 288-295) of VP1 allowed the highly efficient formation of virus-like particles. In contrast, expression level and assembly capacity of fusions to sites 2 (amino acids 222-225) and 3 (amino acids 243-247) were drastically reduced. Immunization of BALB/c mice with chimeric virus-like particles induced a high-titered antibody response against the hantavirus nucleocapsid protein, even in the absence of any adjuvant. The strongest response was observed in mice immunized with virus-like particles harboring 120 amino acids of hantavirus nucleocapsid protein. According to the immunoglobulin subclass distribution of nucleocapsid protein-specific antibodies a mixed Th1/Th2 response was detected. The VP1 carrier itself also induced a mixed Th1/Th2 response, which was found to be reduced in mice immunized with virus-like particles harboring 120 amino acid-long inserts. In conclusion, hamster polyomavirus VP1 represents a promising carrier moiety for future vaccine development.



J Clin Microbiol. 2004 Mar ;42:1322-5 [Pubmed] [Scholar] [Select] [Hide]

First molecular identification of human Dobrava virus infection in central Europe.

Boris Klempa, Morten Schütt, Brita Auste, Milan Labuda, Rainer Ulrich, Helga Meisel, Detlev H Krüger

Viral RNA was amplified by reverse transcription-PCR from a patient suffering from hemorrhagic fever with renal syndrome (HFRS) in Germany. The virus strain could be assigned to the Dobrava hantavirus (DOBV). This is the first molecular identification of human infection by DOBV in central Europe and the first proof that a virus strain related to the DOBV-Aa lineage, carried by Apodemus agrarius rodents, is able to cause HFRS.

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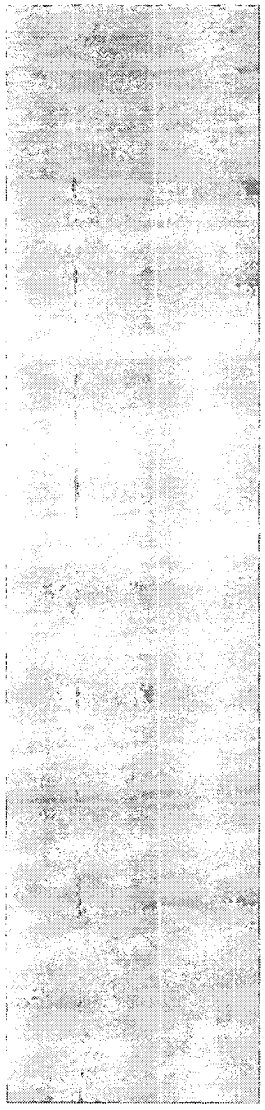
J Clin Microbiol. 2003 Oct ;41:4894-7 [Pubmed] [Scholar] [Select] [Hide]

Occurrence of renal and pulmonary syndrome in a region of northeast Germany where Tula hantavirus circulates.

Boris Klempa, Helga Meisel, Silvana Räth, Jan Bartel, Rainer Ulrich, Detlev H Krüger

Hantavirus species Tula (TULV) is carried by European common voles (Microtus spp.). Its pathogenic potential for humans is unknown. In a rural region of northeast Germany, a 43-year-old man became ill with fever, renal syndrome, and pneumonia. Typing of late acute- and convalescent-phase sera by focus reduction neutralization assay revealed the presence of neutralizing antibodies against TULV. Moreover, we detected TULV genetic material in Microtus arvalis animals that were trapped at places only a few kilometers from the home village of the patient. Phylogenetic analysis of completely sequenced genomic S segments from three virus strains grouped them



within a third genetic lineage of the TULV species. This is the first case of hemorrhagic fever with renal syndrome and pulmonary involvement which can be associated with TULV infection.

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FEBS Lett. 2003 Aug 14;549:157-62 [Pubmed] [Scholar] [Select] [Hide]

Mosaic particles formed by wild-type hepatitis B virus core protein and its deletion variants consist of both homo- and heterodimers.

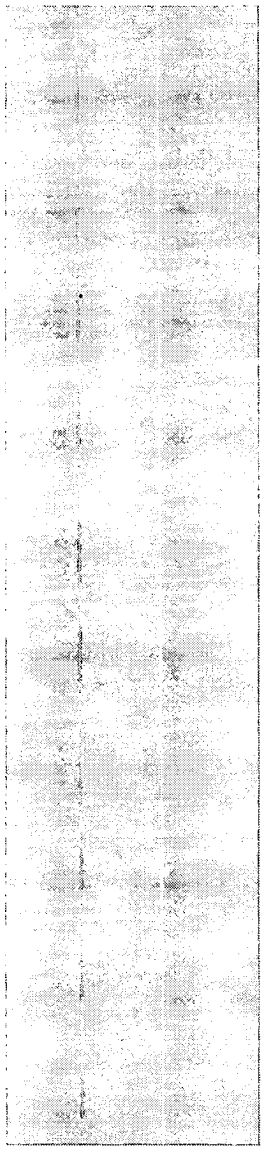
Andris Kazaks, Andris Dishlers, Paul Pumpens, Rainer Ulrich, Detlev H Krüger, Helga Meisel

Co-expression in Escherichia coli of wild-type (wt) hepatitis B virus core protein (HBc) and its naturally occurring variants with deletions at amino acid positions 77-93 or 86-93 leads to formation of mosaic particles, which consist of three dimer subunit compositions. These compositions are wt/variant HBc heterodimers and two types of homodimers, formed by wt HBc or the variant HBc themselves. Mosaic particles were found also when both HBc deletion variants 77-93 and 86-93 were co-expressed in E. coli. These findings are discussed in terms of their significance for hepatitis B virus pathogenesis and prospective use of mosaic particles in vaccine development.

Intervirology. 2002 ;45:340-9 [Pubmed] [Scholar] [Select] [Hide]

Stop codon insertion restores the particle formation ability of hepatitis B virus core-hantavirus nucleocapsid protein fusions.

Andris Kazaks, Sylvie Lachmann, Diana Koletzki, Ivars Petrovskis, Andris Dislers, Velta Ose, Dace Skrastina, Hans R Gelderblom, Ake Lundkvist, Helga Meisel, Galina Borisova, Detlev H Krüger, Paul Pumpens, Rainer Ulrich



In recent years, epitopes of various origin have been inserted into the core protein of hepatitis B virus (HBc), allowing the formation of chimeric HBc particles. Although the C-terminus of a C-terminally truncated HBc (HBc) tolerates the insertion of extended foreign sequences, the insertion capacity is still a limiting factor for the construction of multivalent vaccines. Previously, we described a new system to generate HBc mosaic particles based on a read-through mechanism in an Escherichia coli suppressor strain [J Gen Virol 1997;78:2049-2053]. Those mosaic particles allowed the insertion of a 114-amino acid (aa)-long segment of a Puumala hantavirus (PUUV) nucleocapsid (N) protein. To study the value and the potential limitations of the mosaic approach in more detail, we investigated the assembly capacity of 'non-mosaic' HBc fusion proteins and the corresponding mosaic constructs carrying 94, 213 and 433 aa of the hantaviral N protein. Whereas the fusion proteins carrying 94, 114, 213 or 433 aa were not assembled into HBc particles, or only at a low yield, the insertion of a stop codon-bearing linker restored the ability to form particles with 94, 114 and 213 foreign aa. The mosaic particles formed exhibited PUUV-N protein antigenicity. Immunization of BALB/c mice with these mosaic particles carrying PUUV-N protein aa 1-114, aa 1-213 and aa 340-433, respectively, induced HBc-specific antibodies, whereas PUUV-N protein-specific antibodies were detected only in mice immunized with particles carrying N-terminal aa 1-114 or aa 1-213 of the N protein. Both the anti-HBc and anti-PUUV antibody responses were IgG1 dominated. In conclusion, stop codon suppression allows the formation of mosaic core particles carrying large-sized and 'problematic', e.g. hydrophobic, hantavirus sequences.

Intervirology. 2002 ;45:318-27

Emerging viruses: the case 'hantavirus'.

Rainer Ulrich, Brian Hjelle, Christian Pitra, Detlev H Krüger

This review briefly summarises the recent knowledge about hantavirus infections and raises particular problems in hantavirus research that need further investigation. The following questions are addressed: (i) are hantaviruses distributed worldwide and what leads to new

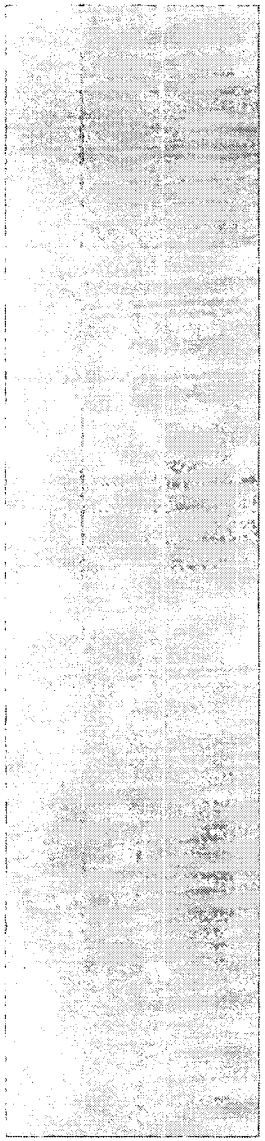
outbreaks, (ii) what is known about hantavirus evolution, (iii) how can hantavirus species be defined, (iv) what are the determinants of hantavirus pathogenesis in humans, and (v) what problems are associated with the development of new vaccines and antiviral therapeutics.

Viral Immunol. 2002 ;15:627-43

Chimeric bacteriophage fr virus-like particles harboring the immunodominant C-terminal region of hamster polyomavirus VP1 induce a strong VP1-specific antibody response in rabbits and mice.

Tatyana Voronkova, Adrian Grosch, Andris Kazaks, Velta Ose, Dace Skrastina, Kestutis Sasnauskas, Burkhard Jandrig, Wolfgang Arnold, Siegfried Scherneck, Paul Pumpens, Rainer Ulrich

The late region of the hamster polyomavirus (HaPyV, former HaPV) genome encodes three structural proteins VP1, VP2, and VP3, where VP1 represents the major capsid protein of 384 amino acids. Screening of sera from HaPyV-infected papilloma-bearing and papilloma-free hamsters demonstrated the immunodominant features of all three capsid proteins. For both groups of hamsters in the C-terminal region of VP1 immunodominant B-cell epitopes were identified in the regions between amino acids 305 and 351 and amino acids 351 and 384. The high flexibility of the C-terminal region of VP1 was confirmed by the formation of chimeric virus-like particles based on the coat protein of the RNA bacteriophage fr which was previously found to tolerate only very short-sized foreign insertions. Phage fr coat protein-derived virus-like particles tolerated the N-terminal fusion of amino acids 333-384, 351-384, 351-374, and 364-384, respectively, of VP1. The induction of VP1-specific antibodies in rabbits and mice by immunization with chimeric virus-like particles harboring amino acids 333-384, 351-384, and 364-384, respectively, of VP1 suggested the immunodominant nature of the C-terminal region of VP1.



J Virol. 2003 Jan ;77:804-9 [Pubmed] [Scholar] [Select] [Hide]

Genetic interaction between distinct Dobrava hantavirus subtypes in Apodemus agrarius and A. flavicollis in nature.

Boris Klempa, Heiko A Schmidt, Rainer Ulrich, Stefan Kaluz, Milan Labuda, Helga Meisel, Brian Hjelle, Detlev H Krüger

Dobrava virus (DOBV) occurs in two different rodent species, Apodemus flavicollis (DOBV-Af) and A. agrarius (DOBV-Aa). We sequenced the S and M genomic segments from sympatric DOBV-Af and DOBV-Aa strains which fell into two distinct genetic lineages. Molecular phylogenetic analyses gave evidence for genetic reassortment between S and M segments of DOBV-Af and DOBV-Aa and indicated homologous recombination events in DOBV evolution. DOBV-Af and DOBV-Aa are distinct but also subject to genetic exchanges that affect their evolutionary trajectories.

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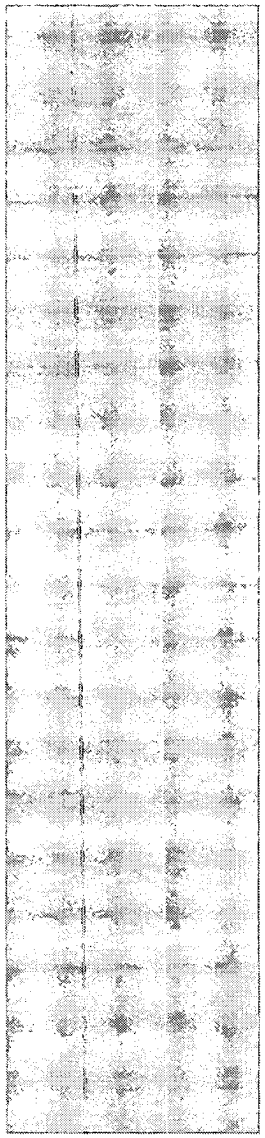
J Virol. 2002 Nov ;76:10724-33 [Pubmed] [Scholar] [Select] [Hide]

Hantavirus infection of dendritic cells.

Martin J Raftery, Annette A Kraus, Rainer Ulrich, Detlev H Krüger, Günther Schönrich

Dendritic cells (DCs) play a pivotal role as antigen-presenting cells in the antiviral immune response. Here we show that Hantaan virus (HTNV), which belongs to the Bunyaviridae family (genus Hantavirus) and causes hemorrhagic fever with renal syndrome, productively infects human DCs in vitro. In the course of HTNV infection, DCs did not show any cytopathic effect and viral replication did not induce cell lysis or apoptosis. Furthermore, HTNV did not affect apoptosis-inducing signals that are important for the homeostatic control of mature DCs. In contrast to immunosuppressive viruses, e.g., human cytomegalovirus, HTNV activated immature DCs, resulting in



upregulation of major histocompatibility complex (MHC), costimulatory, and adhesion molecules. Intriguingly, strong upregulation of MHC class I molecules and an increased intercellular cell adhesion molecule type 1 expression was also detected on HTNV-infected endothelial cells. In addition, antigen uptake by HTNV-infected DCs was reduced, another characteristic feature of DC maturation. Consistent with these findings, we observed that HTNV-infected DCs stimulated T cells as efficiently as did mature DCs. Finally, infection of DCs with HTNV induced the release of the proinflammatory cytokines tumor necrosis factor alpha and alpha interferon. Taken together, our findings indicate that hantavirus-infected DCs may significantly contribute to hantavirus-associated pathogenesis.

cited: 6

Vaccine. 2002 Oct 4;20:3523-31

Yeast-expressed Puumala hantavirus nucleocapsid protein induces protection in a bank vole model.

Ausra Dargeviciute, Katarina Brus Sjölander, Kestutis Sasnauskas, Detlev H Krüger, Helga Meisel, Rainer Ulrich, Ake Lundkvist

Hantaviruses are rodent-borne agents that cause severe human diseases. The coding sequences for the authentic and a His-tagged Puumala hantavirus (PUUV) nucleocapsid (N) protein were expressed in yeast (Saccharomyces cerevisiae). N-specific monoclonal antibodies demonstrated native antigenicity of the two proteins. All bank voles vaccinated with the His-tagged N protein in Freund's adjuvant (n=12) were defined as completely protected against subsequent virus challenge, based on the absence of viral N protein, RNA and G2-specific antibodies. In the group vaccinated with the yeast-expressed authentic N protein in Freund's adjuvant, 2/6 animals were defined as completely protected and 4/6 as partially protected. Moreover, when animals were vaccinated with the His-tagged N protein in an adjuvant certified for human use (alum), all (n=8) were at least partially protected (six completely, two partially). The general advantages of the yeast expression system make the described recombinant proteins promising candidate vaccines against hantavirus infection.

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